Characterization of the human *spr2* promoter: induction after UV irradiation or TPA treatment and regulation during differentiation of cultured primary keratinocytes

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Received May 17, 1990; Accepted July 6, 1990 EMBL accession nos X53064, X53065

ABSTRACT

We have isolated genomic clones from several members of the UV and TPA inducible human *spr2* gene-family in order to analyse the regulation of these genes at a molecular level. From one of these members, the *spr2-1* gene, we have identified and sequenced the regulatory region. By using CAT fusion plasmids and a liposome mediated transfection procedure we show that the isolated promoter region contains all the cis-elements necessary for induced expression after UV irradiation or phorbolester treatment of cultured human keratinocytes. Additionally the *spr2-1* promoter is shown to be regulated aswell during the normal process of keratinocyte differentiation. This makes the *spr2-1* promoter sequence an ideal tool to study the molecular mechanisms by which environmental agents such as UV radiation and chemical tumor promoters interfere with normal gene expression during cell proliferation and differentiation.

INTRODUCTION

UV photons from natural sunlight have been clearly recognized as the responsible component in the induction of many different human skin tumors (1). This relation is especially clear for squamous cell carcinomas and basal cell cancers, both derived from epidermal keratinocytes, where the epidemiological evidence is almost completely consistent with a direct causal role played by UV exposure (2). The induction of skin cancer is a multi-step process involving both genetic and epigenetic changes (3). Whereas the mutagenic action of UV light has been well documented (4) and activated oncogenes have been identified in skin tumors (5,6) the direct influence of UV irradiation on gene expression has only been recognized more recently (reviewed in 7). Here, it will be necessary, in the future, to analyse whether transient changes in gene expression can induce new transcriptional states resulting in epigenetic changes which are thought to play an major role in tumor promotion. As a first approach to such an analysis, it is important to identify the molecular factors involved in the regulation of the normal process of cell proliferation and differentiation and to relate these processes to the molecular changes in gene expression which occur after UV irradiation or treatment with chemical tumor promoters.

Primary cultures of human keratinocytes constitute an ideal system for such an analysis, as these cells retain in culture their normal ability to differentiate and behave in many ways similar to living skin (8). Modification of the ionic Ca^{2+} concentration in the culture medium markedly alters the pattern of proliferation and differentiation (9). While lowering the extracellular concentration of Ca^{2+} below 0.1 mM leads to a marked retardation of differentiation, culturing of cells at physiological concentrations of Ca^{2+} (1−2 mM) induces cell differentiation.

We have previously described the cloning of UV inducible genes by differential screening of cDNA libraries from either UV irradiated or unirradiated keratinocytes (10). The *spr2* gene, identified in such a way, is not only induced by UV irradiation but also by phorbol ester (TPA) treatment (11). Furthermore, the related Spr1 protein was found to be present in higher amounts in differentiating keratinocytes both *in vitro* and *in vivo* (12). Hence, *spr* genes appear to constitute an ideal system to probe, at a molecular level, how UV irradiation and chemical tumor promoters interfere with the normal process of cell proliferation and differentiation.

In this communication, we describe the isolation of the *spr2* promoter region and show that the cloned DNA sequence contains all cis-elements necessary for regulated expression after UV or TPA treatment or during the normal process of cellular differentiation.

MATERIALS AND METHODS

DNA manipulations, bacterial strains and plasmids

DNA cloning techniques were essentially as described by Sambrook *et al.* (13). Genomic fragments containing regions of the *spr2* gene were subcloned from the lambda vector into pIC-20R or pIC-20H (14) and M13mp18 or M13mp19 (13) for sequencing. Plasmid pSG-2 was constructed by inserting the

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promoter region of *spr2-1*, containing the sequences between position −1500 (Hind III) and +14 (Kpn I) upstream of the *cat* gene in pBA-CAT (during the cloning procedure the Kpn I site was deleted). pRSV-CAT contains the promoter of the Rous Sarcoma Virus long terminal repeat inserted before the CAT gene of pBA-CAT. pBA-CAT and pRSV-CAT were a generous gift of Drs. R. Offringa, Leiden. *Escherichia coli* host strains were DH5α (15) for CAT vectors, JM109 (16) for pIC vectors, JM101 (17) for M13 vectors and LE392 (13) for lambda EMBL3. The following DNA fragments were used for probing DNA or RNA blots: an *spr2* specific cDNA probe was derived from clone 930 (11) and contains the whole cDNA insert (excised with RsaI); the *spr2-1* promoter probe consists of the HindIII-KpnI fragment from λspr2A (figure 5). All probes were labelled with α-^{32}P-dCTP by using the random priming method (13).

Cell lines and cell culture

HeLa cells and SV40-transformed xeroderma pigmentosum cells XP_20S-SV complemen-tation group A (18) were maintained in a 1:1 mixture of Dulbecco-Vogt medium (DMEM) and Ham's F10 medium supplemented with 10% fetal calf serum, penicillin and streptomycin. A primary culture of epidermal keratinocytes derived from human foreskin was established as described by Rheinwald and Green (19), with modifications of Ponec et al. (20). Keratinocytes were cultured in the presence of a feeder layer of gamma-irradiated 3T3 mouse fibroblasts in a 3:1 mixture of DMEM and Ham's F12 medium supplemented with 0.4μg/ml of hydrocortisone, 10^{-10} M cholera toxin (Sigma), 10 ng/ml of epidermal growth factor (EGF) (Collaborative Research, Inc.) and 5% fetal calf serum. Medium containing a low calcium concentration (0.06 mM) was prepared as a 3:1 mixture of calcium-free DMEM and standard Ham F12 medium supplemented with 5% chelex-treated fetal calf serum and standard concentrations of hydrocortisone, cholera toxin and EGF. For experiments cells from passages 2 to 4 were used. In the experiments described in this communication hydrocortisone, cholera toxin and EGF were omitted in all cases during and after lipofection.

Genomic library construction and screening

A lambda EMBL3 genomic library of human chromosomal DNA, isolated from peripheral blood of a male CML patient, with an initial complexity of 2×10^6 was a generous gift from Dr. G. Grosveld, Rotterdam (Library 1). From this library a total of 300.000 plaques were screened with the *spr2* specific cDNA probe. A second EMBL3 library, derived from human chromosomal DNA isolated from primary keratinocytes (strain E37), was constructed from MboI partially digested DNA (15−30 kb) and packaged with Gigapack™ II Gold packaging extracts (Stratagene). Library 2 had an initial complexity of 1×10^6 plaques; 200.000 plaques were screened. For screening the libraries were plated at a density of 50.000 plaques per 20×20cm dish (0.6% top agarose in LC medium) and plaques were transfered to nylon filters (Hybond-N, Amersham) essentially as described by the supplier. Prehybridization, hybridization and washing steps were as described by Belt et al. (21).

DNA sequencing

Double strand and single strand sequencing of restriction fragments isolated from genomic clones and subsequently subcloned into pIC and M13 vectors, respectively, were sequenced using α-^{35}S-dATPαS and T7 DNA polymerase (Pharmacia) according to the procedure proposed by the enzyme supplier.

Isolation of cytoplasmic RNA

Gamma irradiated 3T3 cells were first removed by washing twice with PBS contain 0.04% EDTA. Subsequent steps in RNA isolation and all blotting procedures were performed essentially as described before (21).

DNA transfection and CAT assay

HeLa cells and XP_20S-SV fibroblasts were transfected with 20μg pSG-2 per 10cm dish using the DEAE-dextran method (13,22). Primary human keratinocytes were cultured until confluency. The medium was then replaced with DMEM lacking Ca^{2+} for a period of 4 days during which time differentiated cells are shed into the medium (23). Lipofection of the undifferentiated monolayer was performed with the method suggested by the supplier but using 1μg of pSG-2 and 10μg of lipofectin™ (GIBCO-BRL) per 10cm dish. After transfection, cultures were maintained as undifferentiated cells under low Ca^{2+} (0.06mM) conditions or were allowed to differentiate under normal Ca^{2+} (1.8mM) conditions. UV (254nm) irradiation or TPA (12-O-Tetra-decanoylphorbol 13-acetate) (Sigma) treatment was performed 8hr after DEAE-dextran transfection or 16hr after lipofection. 24 or 48 hrs hereafter, cells for scraped from the plates and lysates were prepared in 0.25 M Tris-Cl pH 7.5 by short sonication of the cell pellets (5 seconds at maximum amplitude). The transient expression of the *cat* gene was measured as described by Gorman et al. (24). Plasmids pRSV-CAT and pBA-CAT were included as controls in each experiment.

RESULTS

Kinetics of *spr2* expression after UV irradiation and TPA treatment or during differentiation of primary keratinocytes

In order to analyse the kinetics of *spr2* expression after UV irradiation and TPA treatment, primary keratinocytes were

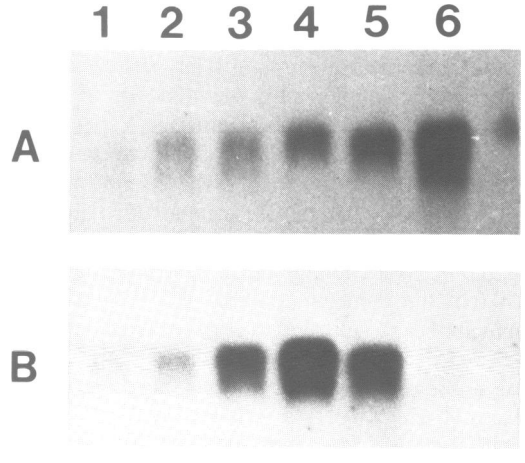

Fig 1: Northern analysis of *spr2* expression in primary keratinocytes. Cultures were irradiated with 40 J/m^2 of UV light (a) or treated with 20ng/ml TPA (b). Total RNA was isolated 0, 3, 6, 12, 24 and 48hr after treatment (lanes 1−6 respectively). Each lane contains 20 μgs of total cytoplasmic RNA. The probe was an *spr2* specific cDNA fragment.

cultured under standard calcium conditions till approximately 80% confluency (Materials and Methods) and were either irradiated with a fluence of 40 J/m^2 of UV light or supplemented with 20 ng/ml of TPA. Total cytoplasmic RNA was isolated at different time intervals hereafter (figure 1). UV irradiation of cultured primary keratinocytes leads to increased cytoplasmic levels of *spr2* mRNA (figure 1a). Although some induction is already observed at 3 hrs after UV, maximum induction is only reached 48 hrs after irradiation indicating a late UV response of *spr2* genes as compared with early UV responsive genes (see discussion). TPA treatment also leads to a clear induction of *spr2* transcription (figure 1b) with a maximum level present at 12 hrs after starting treatment. The decrease in *spr2* expression observed in figure 1b is due to RNA degradation as could be concluded from rehybridization of the Northern blot with a 28S specific ribosomal probe (not shown).

Induction of *spr2* genes occurs aswell during the normal differentiation process of cultured keratinocytes (figure 2). Here, keratinocyte cultures were grown to confluency in standard medium, whereafter differentiated cells were discarded after incubation of the culture in calcium free DMEM (see Materials and Methods). To the undifferentiated monolayer of basal keratinocytes, standard medium (1.8 mM Ca^{2+}) without EGF, hydrocortisone and cholera toxin was added and total RNA was isolated at different time intervals hereafter. The results presented in figure 2 show that *spr2* expression is significantly lower in undifferentiated cells (lane 2) as compared to a mixed population of growing and differentiating cells (lane 1). Furthermore, it is clear that here as well induction of *spr2* genes seems to be a late process as a major increase in *spr2* expression is still observed between 24 and 48 hrs after undifferentiated cultures were permitted to differentiate.

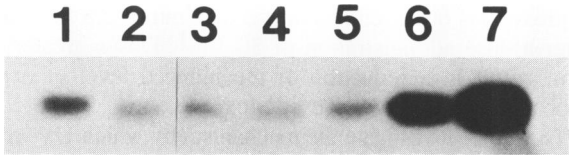

Fig 2: Northern analysis of *spr2* expression during differentiation of primary keratinocytes. Undifferentiated stripped cultures of primary keratinocytes were switched to standard Ca^{2+} (1.8mM) medium and further incubated. Total RNA was isolated 0, 1, 3, 6, 24 and 48hr after the switch (lanes 2−7 respectively). Lane 1 contains total RNA isolated from a mixed (proliferating & differentiating) population of keratinocytes before stripping (see Materials and Methods).

Isolation and characterization of *spr2* genomic clones

From screening of 2 different human chromosomal libraries (Materials and Methods), 3 independent clones, hybridizing specifically to the *spr2* cDNA probe, have been isolated (figure 3): λspr2A and λspr2B from library 1, and λspr2C from library 2. λspr2A and λspr2C are from the same chromosomal localization whereas λspr2B is clearly from a different localization. This observation confirms our previous suggestion that *spr2* might constitute a gene family (11). Such a view is further substantiated by the presence of 2 different *spr2* loci on λspr2A (figure 3). In figure 4 total human DNA from primary fibroblasts was hybridized either with an *spr2* specific cDNA probe or an *spr2-1* derived promoter probe (HindIII-KpnI fragment in figure 5). Whereas the cDNA probe detects at least 7 different loci, the promoter probe hybridizes strongly with one restriction fragment of the size predicted for *spr2-1* (figure 3) and weakly with several (but not all) other loci. Apparently the promoter sequences of the *spr2* gene family have more diverged than the corresponding coding sequences (see discussion).

The spr2-1 gene, localized on λspr2A, has been studied in more detail (figures 5 and 6). The gene consists of 2 exons separated by an intron of approximately 700bp. The first exon (position 627−668 in figure 6) was detected with the aid of a 24 basepair synthetic oligomer corresponding to position 20−44 of the published 930 cDNA sequence (11). The 2nd exon contains the entire open reading frame of the Spr2-1 protein (position 836−1051 in figure 6). The deduced amino-acid sequence of the Spr2-1 protein differs in 4 positions (C45, Y54, S65 and Y67) from the *spr2* sequence derived from the 930 cDNA clone (11). C45 and Y54 are however conserved in cDNA clones 174N and 1292 (11), which confirms the previously observed heterogeneity inside the *spr2* gene-family. The sequences of the intron boundaries of *spr2-1* conform to the established consensus (25) and the position is conserved in both the *spr2-2* and *spr2-3* genes (unpublished results).

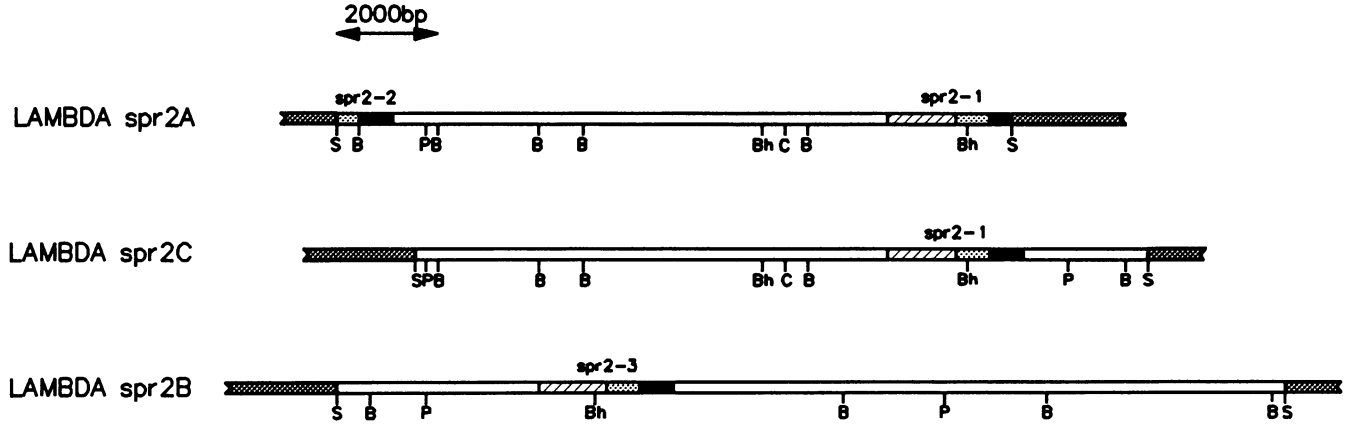

Fig 3: Restriction map of *spr2* hybridizing lambda EMBL3 clones. The different *spr2* loci are indicated and the following symbols have been used: shaded area = promoter region; stippled area = introns; black boxes = exons; grey areas = lambda arms; the following restriction enzymes have been used: B: BglII; Bh: BamHI; C: ClaI; P: PstI; S: SalI;

Characterization of the *spr2-1* promoter

The entire promoter region of the *spr2-1* gene was isolated on a 1500 basepair long HindIII-KpnI fragment from λspr2A (figure 5). In figure 6 the nucleotide sequence of the proximal 600 nucleotides of this promoter region is represented. The position of the transcriptional start site (+1) was inferred from the analysis of 5 independent cDNA clones (930, 1137, 174N, 375 and 1480 in reference 10). 27 nucleotides ahead of this sequence a TATA box consensus is found. At position −195, the sequence TGAGTCA was observed. This sequence has previously been described as a recognition and binding site for transcription factor AP-1 (26) and was shown to be essential for the induction of several genes (e.g. collagenase and stromelysin) after UV irradiation or TPA treatment (27).

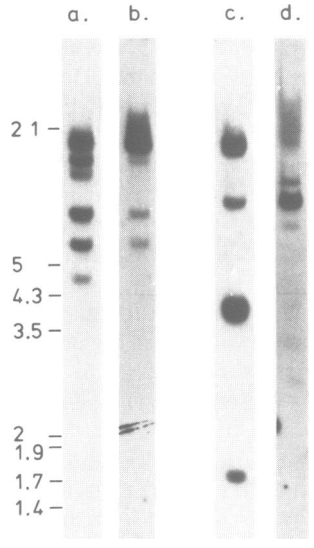

Fig 4: Southern blot analysis of the *spr2* gene family: each lane contains 10 μgs of human DNA (isolated from primary diploid fibroblasts); lanes a) and b): DNA digested with PstI; lanes c) and d): DNA digested with BglII; lanes a) and c) were hybridized with an *spr2* specific cDNA probe (Materials and Methods); lanes b) and d) were hybridized with an *spr2-1* specific promoter probe (HindIII-KpnI fragment in figure 3). Lambda restriction fragment markers are indicated in the left margin.

Transfection of primary human keratinocytes

Primary cultures of human keratinocytes undergo a well-defined program of terminal differentiation which can be regulated by the concentration of Ca^{2+} ions in the culture medium. Consequently, the classical calcium transfection procedure (28) can not be used for the introduction of exogenous DNA into these cells as high calcium concentrations have a main impact on the physiology of these cells. This can especially be a problem if subsequent expression studies are planned. The DEAE-dextran procedure (13,22) which is used by most researchers in transient CAT assays turned out to be inefficient for primary human keratinocytes (results not shown). We have, however, successfully transfected human keratinocytes by using a liposome mediated transfection procedure (29). A proliferating monolayer of primary keratinocytes, obtained after stripping of mixed cultures (Materials and Methods) was used as acceptor for exogenous DNA. The option is then open to maintain the culture in the proliferating form (low Ca^{2+} conditions = 0.06 mM) or to induce differentiation by increasing the Ca^{2+} concentration to 1.8 mM. Our results (see below) suggest that lipofection is a gentle and reliable means to introduce foreign DNA into primary human keratinocytes without any obvious stress to the cells.

Expression of the *spr2-1* promoter in primary keratinocytes and different cell lines

The promoter region of the spr2-1 gene can direct the expression of the CAT reporter gene when transfected into a primary keratinocyte culture (figure 7). After UV irradiation, increased expression is observed reaching a maximum (2.8 fold induction) after a dose administration of 30 J/m^2. Doses greater than 30 J/m^2 result in a reduction of the induced level of expression probably due to excessive cytotoxicity.

To further investigate the mechanism by which UV irradiation induces the expression of the *spr2-1* promoter, pSG-2 was introduced into the $XP_2$0S-SV cell-line and into HeLa cells. An important difference between these two cell-lines is the deficiency in DNA excision-repair of $XP_2$0S-SV, as compared to the repair proficiency of HeLa cells. Consequently, if DNA damage is the inducing trigger, one would expect that much lower UV doses would be required in the case of the $XP_2$0S-SV cell-line in order to get the same level of induction of the *spr2-1* promoter as in HeLa cells. Results in figure 8 show that this is indeed the case.

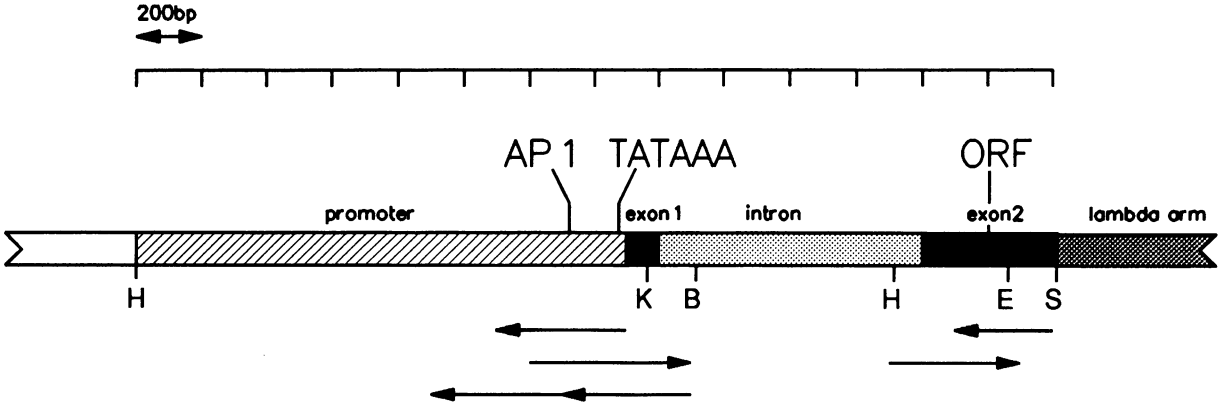

Fig 5: Restriction map of the *spr2-1* locus. The different symbols are described in figure 3. E: EcoRI; H: HindIII; K: KpnI; ORF: open reading frame; binding sites for transcription factor AP-1 and RNA polymerase (TATAAA) are indicated; arrows indicate the sequenced sections represented in figure 5.

A UV fluence of 1 J/m^2 is sufficient for an 3.9 fold induction of the *spr2-1* promoter in XP$_2$OS-SV, whereas 10 J/m^2 are needed for an 3.5 fold induction in HeLa cells.

The results represented in figure 9 show that the isolated promoter sequence contains aswell the regulatory elements responsible for TPA induction in HeLa cells. Maximum stimulation was reached with cells treated with 20 ng/ml TPA. The same concentration of TPA resulted aswell in a significant induction of *spr2-1* transcription in primary keratinocyte cultures (figure 10). As with UV light, the regulation by TPA does not seem to be cell type specific.

We have shown by Northern analysis (see above), that the *spr2* gene family is regulated during the normal differentiation process of human keratinocytes. To investigate whether our isolated promoter sequence contains all cis-acting elements necessary for this regulation, pSG-2 was transfected into an undifferentiated monolayer of basal keratinocytes. When these cells were permitted to differentiate in medium containing 1.8 mM Ca^{2+} *spr2* promoter expression was induced 18 fold as compared to a parallel culture which was maintain in medium which differed only by a low calcium concentration (0.06 mM) (figure 11). It is interesting, in this respect to note that in general the basal level of expression of *spr2-1* in non-differentiating XP$_2$OS-SV and HeLa cells is several fold lower than in keratinocyte cultures grown under standard conditions.

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   1  AGGTAAGAAGAAAATTCAGCTCGAACATCGGATCAGGTGGCACAATGCGGTCAATGCCTG

  61  CAAACTCAGGGTAAGTATTATTCTCCCTGTTTACAGTTCCGTGGAGGAGAAGTGACTTGC

 121  CTGTGGTCATACAACAGAGCAAAGAAAAGGCTTGAGCTAGAACTCAGGCCTTTGTTAGGT

 181  CTCCCCTTCCTCCTAGCACATTGGCAAATTGCATGAGGAAAGTAGAGGTACAGTTGAGTT

 241  CATGTACAACAATAAGGCATTCAGGTAAAGTGAATGAGGCAGAAGTTTTATGATTTAGG

 301  GAAGGTGTAAGACAGGAAAATATCTTTGTTCCCAATTAAGAAAGAGATCCCTTGACCATC

 361  AGTTAGAGATTCCCCCAAGTCCCTCTTTGCCATAAGTCACTGAAACTGAGATCCAAGGCA

                AP-1
 421  TGGCTTCTGTGAGTCAGGAGAGCTTAACCCAGAGGAGAGATTTCAGAACAGGATATTTCC

 481  TATTTTGAGTATCCTGCTCATGCCAGTCATGGATAAATTTGCATCTGGCTTAAGAAATTA

 541  CTGGATCAGCATTGTTTTGGGTAGTTTCACTTCCTGCTGGGTGGGGTAGCAGGCTCTATA

                    +1
 601  AAGAGATCCTCTGCTGCACGACTCTTAAACCCCTGGTACCTGAGCACTGATCTGCCTTGG

            5' splice site
 661  AGAACCTGgtgagtcggcttccttgagttcctctgttctttgtgccctgaaatgttgagt

 721  ttaatctgaatatggcaagtttggtggatcc -- 700-800 bp -- aagctttggct

                              3' splice site
 763  tctctctctggaggattccttccacgaacactgttgtaatcatttctttcagATCCTGAG

 823  GACTCCAGCAGGATGTCTTATCAACAGCAGCAGTGCAAGCAGCCCTGCCAGCCACCTCCT
                M  S  Y  Q  Q  Q  Q  C  K  Q  P  C  Q  P  P  P   16

 883  GTGTGCCCCACGCCAAAGTGCCCAGAGCCATGTCCACCCCCGAAGTGCCCTGAGCCCTGC
      V  C  P  T  P  K  C  P  E  P  C  P  P  P  K  C  P  E  P  C   36

 943  CCACCACCAAAGTGTCCACAGCCCTGCCCACCTCAGCAGTGCCAGCAGAAATATCCTCCT
      P  P  P  K  C  P  Q  P  C  P  P  Q  Q  C  Q  Q  K  Y  P  P   56

1003  GTGACACCTTCCCCACCCTGCCAGTCAAAGTATCCACCCAAGAGCAAGTAACAGCTTCAG
      V  T  P  S  P  P  C  Q  S  K  Y  P  P  K  S  K  *            72

1063  AATTCATCAGGACCAAGAAAGGATAAGGATATTTGGCTCACCTCGTTCCACAGCTCCACC

1123  TTCATCTTCTCATCAAAGCCTACCATGGATACACAGGGAGCTTCTTTCTCCTTAGCCAGT

1183  AATCTGCCCATGATGATC
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Fig 6: Nucleotide sequence of the *spr2-1* locus: AP-1 binding site, TATA box and transcriptional start site (+1), aswell as a repetitive sequence (position 140−280) in the promoter region, are represented in a bold letter type. The intron is represented in low case letters and the conserved 5′ and 3′ sequences have been highlighted. The aminoacid sequence of the Spr2-1 protein is represented in the one letter code. The sequencing strategy is shown in figure 5.

DISCUSSION

We have isolated the regulatory elements of one member of the *spr2* gene family and shown that these sequences contain all the cis-elements necessary for stimulated expression after UV and TPA treatment or during the normal process of differentiation in cultured primary keratinocytes. Induction of *spr2-1* by UV and TPA does not seem to be cell type specific or dependant

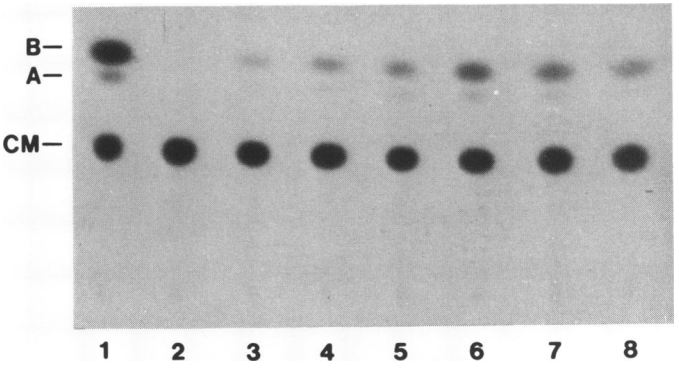

Fig 7: UV induction of *spr2-1* in primary keratinocytes. Control transfections were performed with pRSV-CAT (lane 1) and pBA-CAT (lane 2). Lanes 3−8 show CAT expression driven by the *spr2-1* promoter (on pSG-2) 48 hrs after 0, 10, 20, 30, 40 and 50 J/m^2 of UV irradiation respectively. CAT activity was determined with 30 μg of cell extract in a 1 hour reaction and was quantified after scanning the autoradiogram with an LKB Ultroscan Laserscanner. The percentage of total chloramphenicol converted to the acetylated form was in lanes 1 to 8 respectively: 51, 0, 11, 17, 20, 31, 23, 19; CM = chloramphenicol, A = 1-acetate chloramphenicol and B = 3-acetate chloramphenicol.

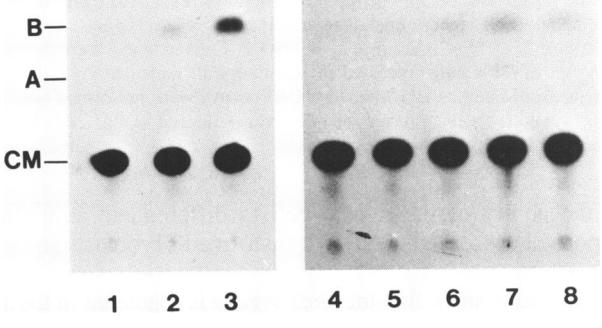

Fig 8: UV induced DNA damage is responsible for *spr2-1* induction. The XP$_2$OS-SV cell line (lanes 1−3) and HeLa cells (lanes 4−8) were transfected with pSG-2 using the DEAE-dextran procedure. Lanes 1−3 show CAT expression 48hrs after respectively 0, 0.5 and 1 J/m^2 of UV irradiation in XP cells whereas lanes 4 to 8 show CAT expression after respectively 0, 5, 10, 15 and 20 J/m^2 of UV irradiation in HeLa cells. CAT activity was measured with 35 μg of cell extract in a 6 hr assay. The following conversion percentages (refer to the legend of fig. 7) were obtained in lanes 1 to 8 respectively: 8, 16, 31, 2, 3, 7, 11, 9;

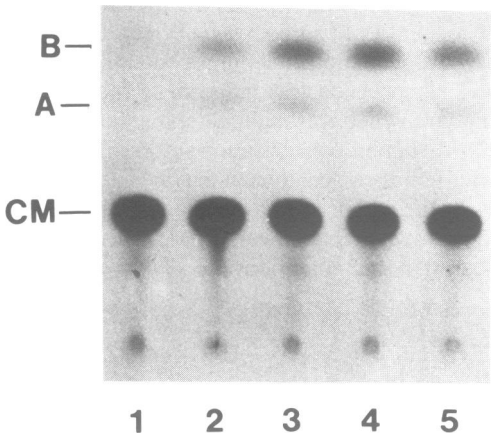

Fig 9: Induction of *spr2-1* promoter activity after TPA treatment. HeLa cells were transfected with pSG-2 by using the DEAE-dextran method. Lanes 1−5 show CAT expression 48hr after treatment with 0, 5, 10, 20 and 40 ng/ml TPA respectively. CAT activity was measured with 35 μg of cell extract in a 6 hr assay: the following conversion percentages (legend of fig. 7) were determined in lanes 1 to 5 respectively: 2, 10, 17, 19, 18;

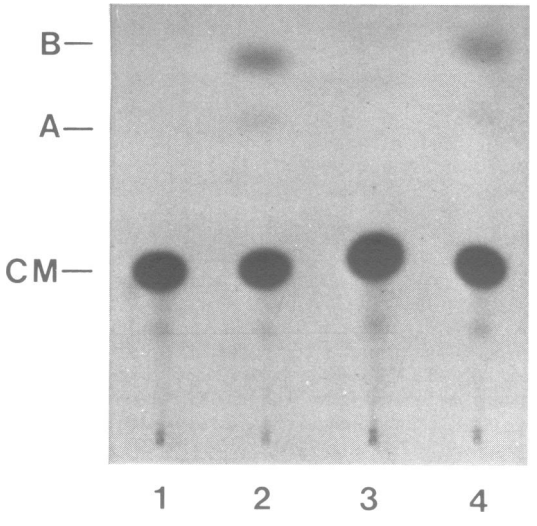

Fig 10: Induction of *spr2-1* transcription after TPA treatment in primary keratinocytes. Cells were lipofected with the following plasmids: lane 1: pBA-CAT; lane 2: pRSV-CAT; lanes 3−4: pSG-2. Cells in lane 4 were treated with 20 ng/ml of TPA and processed in parrallel with untreated cells (lane 3) as described in Materials and Methods. CAT assays were performed with 10 μg of cell lysate for 1 hr. Conversion percentages (legend of fig. 7) in lanes 1 to 4 were 0, 15, 1.5 and 10,5 respectively.

on the ability of the treated cell to differentiate as a similar response is observed in SV40 transformed fibroblasts and HeLa cells (figures 8 and 9).

Our results show that the *spr2-1* gene is regulated in the same way as the whole *spr2* gene family (measured by Northern analysis). However, it has yet to be determined whether all *spr2* loci are regulated similarly. Recently we have isolated the promoter region of the *spr2-3* gene (figure 3) and DNA sequencing showed that this promoter region has 74% homology with the corresponding *spr2-1* locus. These results confirm the results obtained by Southern blotting described in figure 6. It is interesting to note that this rather weak homology between two

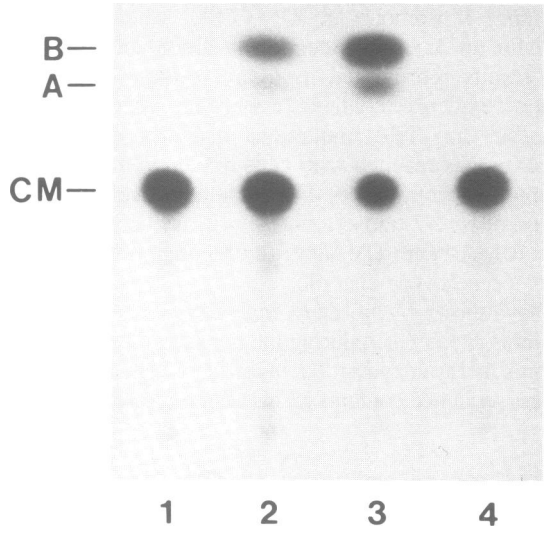

Fig 11: Induction of spr2-1 promoter activity during normal differentiation of primary keratinocytes. Stripped cultures were lipofected with pSG-2 (lane 1 and 2), pRSV-CAT (lane 3) or pBA-CAT (lane 4) whereafter the cells were kept in either low or normal Ca^{2+} medium. In lane 1 spr2-1 promoter expression was monitored under low Ca^{2+} (0.06mM) conditions whereas lane 2 shows the activity of the same promoter under normal physiological calcium conditions (1.8mM). Control transfections (lanes 3−4) were kept under normal calcium conditions after lipofectin treatment. The assay was performed with 9 μg of lysate for 6 hrs. Conversion percentages in lanes 1 to 4 determined as described in the legend of fig. 7 were respectively 2, 36, 57, 0;

promoter sequences inside a same gene-family is due to an alternation of blocks of very high homology (10−20 basepairs) with regions of low homology, suggesting a modular structure of *spr2* promoters as found aswell in other eucaryotic regulatory regions (30). One of the main regions of homology includes a potential AP-1 binding site which has been shown to be involved in the regulation of other UV and TPA inducible genes (7,27,31). Other conserved regions may contain further cis-acting sequences possibly involved in the regulation of these genes.

The finding that at least two members of the *spr2* gene-family contain a putative AP-1 binding site suggests that the proto-oncogenes *c-fos* and *c-jun* might be involved in the regulation of these genes (32). The kinetic analysis performed in figure 1 suggests that induction of *spr2* genes occurs gradually with a first level of induction between 3−6 hours after irradiation, an intermediate level between 12−24 hours and a very high level 48 hours after administration of a UV-dose of 40 J/m^2. If Fos and Jun, which have been shown to be activated very quickly (within two hours) after UV irradiation (7,33), are actually involved in the UV induced expression of *spr2*, than our kinetic analysis might indicate that these oncogenes are not solely involved in the regulation of these genes but that other signal transduction cascades activated by UV light might aswell be involved. The *spr2-1* and *spr2-3* promoters do not contain sequences resembling an NF-$\varkappa$B binding site or a serum responsive element (SRE). These two cis-acting promoter elements have been recently implicated in the regulation of other UV induced genes (34).

We have shown that the UV-enhanced expression of the *spr2-1* gene is due to DNA damage of the kind not repaired in cells from patients with xeroderma pigmentosum (complementation group A). This could be concluded from the observation that in XP-A cells a 10−20 fold lower UV dose was needed in order

to reach a similar increase in expression as in repair proficient HeLa cells (figure 8; table 2) As a dose of 1 J/m^2, which results in a 4 fold induction of the *spr2-1* promoter in a mass culture of XP cells introduces approximately 1 thymidine dimer into 100,000 base pairs of DNA, it should be clear that at such a frequency the probability is very low that the promoter region of the target gene has absorbed UV radiation itself and has been activated in this way. Rather, this evidence suggests that the presence of DNA damage somewhere in the genome activates a signal transduction cascade which in turn stimulates expression of the UV responsive *spr2* gene-family. Similar conclusions have recently been drawn by Herrlich's group at the University of Karlsruhe (34).

Our results show that the expression of the *spr2-1* promoter is also induced by the tumor promoting agent TPA. As UV light has aswell tumor promoting activity (35,36), it is tempting to speculate that *spr* genes might be directly involved in tumor promotion. More research is however needed to establish the precise function of these genes during normal development and carcinogenesis. It will be important to analyse whether in the case of *spr2-1* the same cis-acting promoter elements are involved in both UV and TPA induced regulations as has been found for the collagenase gene (28,34). This is particularly interesting as in the case of the related *spr1* and *spr3* genes this overlap between UV and TPA induction does not seem to exist (unpublished results).

Finally, the isolated *spr2-1* promoter is shown to contain all sequences necessary for regulated expression during the normal differentiation process of cultured primary keratinocytes. Hence, deletion mapping and footprinting of the *spr2-1* promoter region can now be used in order to identify the cis-acting elements aswell as the corresponding transfactors involved in the normal regulation of these genes. This knowledge will be essential for understanding the molecular mechanisms by which environmental agents such as UV light or tumor promoting chemicals interfere with the normal process of cell proliferation and differentiation.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Maja Ponec (Dermatology, AZL, Leiden) for her help with keratinocyte cell cultures, Prof. Peter Herrlich (Karlsruhe) for his interest and helpful suggestions and Peter Belt, Vijai Sardjoe Missier and Remond Fijneman for their contribution in several experiments. This research was supported by the J.A. Cohen Institute for Radiopathology and Radiation Protection (IRS) and by the Foundation for Scientific Research in the Netherlands (NWO-SON).

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